

UCLA

UCLA Previously Published Works

Title

Mast cell clones: a model for the analysis of cellular maturation.

Permalink

<https://escholarship.org/uc/item/4ch942fq>

Journal

The Journal of cell biology, 95(2 Pt 1)

ISSN

0021-9525

Authors

Galli, SJ
Dvorak, AM
Marcum, JA
et al.

Publication Date

1982-11-01

DOI

10.1083/jcb.95.2.435

Peer reviewed

Mast Cell Clones: A Model for the Analysis of Cellular Maturation

STEPHEN J. GALLI, ANN M. DVORAK, JAMES A. MARCUM, TERUKO ISHIZAKA, GARY NABEL, HAROUT DER SIMONIAN, KATHRYN PYNE, JEROLD M. GOLDIN, ROBERT D. ROSENBERG, HARVEY CANTOR, and HAROLD F. DVORAK

Departments of Pathology and Medicine, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachusetts 02215; Whitaker College of Health Science, Technology, and Management, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; Departments of Medicine and Microbiology, Johns Hopkins University School of Medicine and the Good Samaritan Hospital, Baltimore, Maryland 21239; and Departments of Cell Biology and Pathology, Sidney Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02215

ABSTRACT Cloned mouse mast cells resemble, by ultrastructure, immature mast cells observed in vivo. These mast cell clones can be grown in the absence of any other cells, facilitating direct investigations of their biochemistry and function. We find that cloned mast cells express plasma membrane receptors ($Fc\epsilon R$) that bind mouse IgE with an equilibrium constant (K_A) similar to that of normal mouse peritoneal mast cells. In addition, cloned mast cells do not display detectable Ia antigens and cannot enhance Ig secretion when added to lymphocyte cultures or mediate natural killer lysis. In the presence of 1 mM sodium butyrate, cloned mast cells stop dividing and acquire abundant electron-dense cytoplasmic granules similar to those of mature mast cells. Their histamine content increases concomitant with cytoplasmic granule maturation and may exceed that of untreated mast cells by 50-fold. Unlike peritoneal mast cells, cloned mast cells incorporate $^{35}SO_4$ into chondroitin sulfates rather than heparin. These findings demonstrate that, unlike fully differentiated mouse peritoneal mast cells, cloned immature mouse mast cells contain no heparin and low levels of histamine. In addition, they establish that high-affinity $Fc\epsilon R$ are expressed early in mast cell maturation, well before completion of cytoplasmic granule synthesis and mediator storage.

We have previously reported methods to clone mast cells with normal karyotypes from mouse hematopoietic tissue in vitro (30). Our cloned mast cells contain less histamine than normal mouse peritoneal mast cells and resemble immature mast cells by morphology. Others have described similar findings with uncloned cells (22, 32, 38, 42, 43, 51, 53). Although some mast cells synthesize heparin when fully differentiated (23, 54), certain mast cell tumors are devoid of heparin and incorporate $^{35}SO_4$ into chondroitin sulfate exclusively (18). Histochemical evidence suggests that normal immature connective tissue mast cells (6) and the mast cells in the intestinal lamina propria of rodents (25, 50) also may synthesize glycosaminoglycans other than heparin, but this notion has not been confirmed directly.

In this report, we show that cloned mast cells closely resemble, by ultrastructure, immature mast cells found in vivo.

Cloned mast cells incorporate $^{35}SO_4$ preferentially into chondroitin sulfates, confirming histochemical evidence that immature mast cells contain little heparin. In addition, cloned mast cell proliferation, cytoplasmic granule synthesis, and mediator storage can be modulated in vitro, permitting direct analysis of mast cell maturation.

MATERIALS AND METHODS

Antisera

Lyt-1.2 and Lyt-2.2 antisera, prepared as described (45), were kindly donated by F. W. Shen¹; monoclonal antibody against Thy-1.2 (mc- α -Thy-1.2) was donated by Ed Clark²; and mc- α -Lyt-1 and mc- α -Lyt-2 were gifts from J.

¹ Memorial Sloan-Kettering Cancer Center, New York, NY

² University of Washington, Seattle, WA

Ledbetter and L. Herzenberg³. Mast cells were examined for Ia antigens using ATH- α -ATL alloantisera (16) or mc- α -Ia (10-3.6, reference 35) generously provided by John Freed and J. M. Kupinski⁴.

Mast Cells

Procedures for the isolation, growth, and cloning of mast cells from mouse hematopoietic tissue have been previously described (30). Briefly, cells from the liver of a 13-d-old mouse fetus from one A/J female mated with a C57BL/6 male were incubated in Dulbecco's modified Eagle's medium (DME) with 4% heat-inactivated fetal calf serum (FCS), 5×10^{-5} M 2-mercaptoethanol and 2 mM glutamine (DME-FCS) conditioned by concanavalin A (Con A)-activated BALB/c spleen cells (Con A-CM). 10 d later, cells were distributed at limiting dilutions in wells containing irradiated (2,000 R) syngeneic bone marrow cells. Colonies appeared at 10-14 d with a cloning efficiency of ~10%. All colonies grown under these conditions were composed of cells that resembled mast cells by light microscopy because of their prominent metachromatic cytoplasmic granules. All colonies expressed the Ly 5⁺ Thy 1⁻, Ly 1⁻2⁻ phenotype of surface membrane glycoproteins. No growth of mast cells was observed from preparations of irradiated bone marrow cells. Mast cell lines were cloned by micromanipulation (30) and have been designated CLMC/1-14. The cloned cells have normal karyotypes and can be cultivated in large numbers ($>10^6$) with doubling times in Con A-CM of 36-48 h. Clones are stable for at least 24 mo, either alone or with an irradiated spleen cell feeder layer, although doubling times of cells maintained in Con A-CM may progressively increase. Using similar conditions, mast cell colonies also may be isolated from adult mouse spleen or bone marrow cells. Treatment of spleen or marrow cells with antiserum to Thy 1.2 and complement facilitates growth of mast cell colonies, which by electron microscopy contain >95% (usually >99%) mast cells after 4-6 wk of culture. We have successfully maintained our cultures with medium conditioned by Con A-stimulated splenocytes (Con A-CM), cloned Ly 1⁺2⁻ inducer T lymphocytes (31), or WEHI-3 cells (32). All the cells described in this report were maintained in Con A-CM unless otherwise specified. The active growth factor in the supernatant of cloned Ly 1⁺2⁻ inducer T lymphocytes has been purified to apparent homogeneity. It has a pI of ~6.0 and a *M_r* of 45,000 (30) (Nabel et al., manuscript in preparation).

Effect of Sodium Butyrate on Mast Cells

Mori et al. reported that p-815 murine mastocytoma cells, ordinarily devoid of cytoplasmic granules, underwent granulogenesis when exposed to sodium butyrate over 3-4 d in vitro (26). Butyrate-induced granulogenesis was accompanied by a prolongation of mastocytoma doubling time and an increase in cell size, changes that suggested a differentiation effect. We evaluated whether sodium butyrate might exert similar effects on our mast cell clones. In these experiments, mast cells (1×10^5 cell/ml) were cultured in Con A-CM containing 10-20% medium conditioned by Ly 1⁺2⁻ inducer T lymphocytes (31) supplemented with various concentrations of sodium butyrate (Pfaltz and Bauer, Stamford, CT) for 4 d. In some experiments, 1-ml aliquots of cells were cultured in 24-well plates (Costar, Data Packaging, Cambridge, MA). In other experiments, 5- to 10-ml aliquots of cells were seeded into 25 cm² tissue culture flasks (Falcon Labware, Oxnard, CA). The medium, including butyrate, was replaced after 48 h of culture. After 4 d, cells were recovered for electron microscopy and for determination of cell number and histamine content.

Transmission Electron Microscopy (TEM) and Autoradiography

Cells were fixed in suspension for 1 h at room temperature in a mixture of paraformaldehyde and glutaraldehyde (9), were washed twice in 0.1 M sodium cacodylate buffer, pH 7.4, and were centrifuged through soft agar in a microfuge. The agar-embedded pellets were then processed for electron microscopy either by the osmium-potassium ferrocyanide (OPF) or osmium-collidine uranyl en bloc (OCUB) techniques (9). They were then dehydrated in a graded series of alcohols and infiltrated and embedded in a propylene oxide-Epon sequence. For autoradiography, ³⁵S-labeled cells (see below) were fixed and processed by the OCUB techniques as above. Thin Epon sections were cut with an LKB 5-ultratome (LKB Instruments, Rockville, MD), were placed on copper grids and looped with Ilford L-4 emulsion, exposed for up to 4 mo under desiccated conditions at 4°C, developed with Microdol X (Kodak), and stained lightly with lead citrate. Sections were examined in a Philips 400 electron microscope.

Histamine Determination

Histamine was routinely measured fluorometrically with an Autoanalyzer II

³ Stanford University, Stanford, CA

⁴ Johns Hopkins University, Baltimore, MD

(Technicon Instruments Corp., Tarrytown, NY) equipped to detect histamine in the 0.5-10 ng/ml range (46). The histamine content of some samples was also determined before and after treatment with diamine oxidase by the isotopic-enzymatic method (44), with similar results.

IgE Receptors

The methods used to enumerate and characterize the binding properties of Fc ϵ R have been described in detail (17, 48). Briefly, washed cells were resuspended in MEM or RPMI-1640 medium with 0.01 M EDTA and 10% FCS, pH 7.4, at $1-30 \times 10^5$ cells/ml. Cells in 0.4 ml received 0.05 ml of additional medium or medium containing 10 mg/ml noniodinated, affinity purified mouse monoclonal IgE antibody from hybridoma H 1 DNP- ϵ -26 (20). After 15 min at 37°C, ¹²⁵I-IgE, $\geq 80\%$ bindable to the rat basophilic leukemia cell line (16), at 100 μ g/ml was added in 0.05 ml for 90 min at 37°C with constant shaking. Duplicate determinations of binding of ¹²⁵I-IgE to cells were made by layering 0.2-ml aliquots of cells onto 0.2 ml of heat-inactivated FCS and centrifuging the tubes in a microfuge. The radioactivity in the cell pellets was measured in a gamma counter and the number of ¹²⁵I-IgE molecules bound per cell calculated according to the formula:

$$\frac{\text{Mean cpm in cells without excess noniodinated IgE} - \text{Mean cpm in cells with excess noniodinated IgE (nonspecific binding)}}{\text{cpm of } 1 \text{ ng } ^{125}\text{I-IgE}} \times \frac{3.2 \times 10^9}{\text{cell number}}$$

The forward rate constant (*k*₁) of IgE binding to MC/9 was measured by incubating prewarmed cells (1.3×10^6 /ml) with 3 μ g/ml of ¹²⁵I-labeled mouse monoclonal IgE at 37°C in Dulbecco's modified Eagle's medium containing 10% FCS and 0.01 M EDTA with constant shaking. The binding reaction was stopped at various intervals by adding a 133-fold excess of unlabeled IgE. Cells in control tubes were incubated with a 133-fold excess of unlabeled IgE before addition of ¹²⁵I-IgE. Nonspecific binding of radioactivity to control cells was subtracted from experimental tubes to determine specific binding. The number of IgE receptors per cell was determined separately by incubating the cells with 10 μ g/ml of ¹²⁵I-IgE for 90 min as described above. To measure the dissociation rate (*k*₋₁) of IgE bound to MC/9, cells (1.3×10^6 /ml) were incubated with 3 μ g/ml of ¹²⁵I-labeled mouse monoclonal IgE at 37°C for 1 h in Ly 1⁺2⁻ T lymphocyte-conditioned Dulbecco's modified Eagle's medium as described above. Cells were then divided into two tubes, centrifuged, and resuspended in fresh medium. A 133-fold molar excess of unlabeled IgE or an equal volume of culture medium was added to each tube at *t*₀. The cells were incubated at 37°C with constant slow rotation and residual cell-bound ¹²⁵I-IgE was determined at various intervals.

Synthesis of Sulfated Glycosaminoglycans

Procedures reported previously for the characterization of guinea pig basophil glycosaminoglycans were used with minor modifications (36).

LABELING OF MAST CELLS WITH [³⁵S]SULFATE: Briefly, cloned mast cells (CLMC/9: 5×10^6 cells in 5 ml DME 4%-FCS containing 25% (v/v) Con A-CM) were labeled with 1 mCi sodium [³⁵S]sulfate (New England Nuclear, NEX-041) for 4-21 h at 37°C. After labeling, cells were washed three times in DME, an aliquot was taken for TEM autoradiographs (see above and reference 36), and the remainder was stored at -70°C for biochemical analysis. In addition to CLMC/9, we also labeled uncloned populations of mast cells derived from adult spleen (line 1) or bone marrow (line 2) maintained in DME-4% FCS supplemented with medium conditioned by WEHI-3 cells (32) and an uncloned population of mast cells derived from bone marrow (line 3) maintained in 10% ConA-CM conditioned by cloned Ly 1⁺2⁻ T lymphocytes. These preparations (>99% mast cells by TEM) were labeled for 12-18 h as above except that the DME lacked unlabeled SO₄ during the incubation period. In some experiments, mast cells were maintained with or without 1 mM sodium butyrate for 4 d before labeling with ³⁵SO₄ as above.

Normal mast cells were purified from peritoneal cells of 6-wk-old CBA/J mice (Jackson Laboratories, Bar Harbor, ME) over a metrizamide gradient as previously described (48). After the metrizamide gradient, the cells were washed twice in Minimal Essential Medium with Hanks' salts (Gibco, Grand Island Biological Co., Grand Island, NY) with PIPES buffer (0.47 g/liter) and 4% FCS. Cells (55-80% mast cells) were labeled with sodium [³⁵S]sulfate for 3-4 h in DME without unlabeled SO₄ supplemented with 4% FCS.

CHARACTERIZATION OF GLYCOSAMINOGLYCANS: Glycosaminoglycans are long chain polysaccharides consisting of repeating disaccharide units. In mast cell granules, glycosaminoglycans are linked to a protein core (23, 54) to form proteoglycans. The ³⁵S-labeled glycosaminoglycans of cultured mast cells were characterized by DEAE chromatography and sequential hydrolysis with

* 1 ng IgE = 3.2×10^9 molecules.

highly purified mucopolysaccharidases of established substrate specificities.

Thawed aliquots of frozen ^{35}S -labeled cells were heated in boiling water for 5 min to inactivate endogenous degradative enzymes (34), and then exhaustively digested with pronase P, which solubilized >90% of incorporated [^{35}S]sulfate. Glycosaminoglycans were chromatographed on DEAE-Sephadex A-25 (0.8×6.9 cm column) previously equilibrated with 0.15 M NaCl, 0.01 M Tris, pH 7.5. Fractions were eluted in 0.5-ml volumes with a two-stage linear gradient of NaCl (0.15–2.0 M; 2.0–4.0 M) (36). Glycosaminoglycans were then incubated with specific mucopolysaccharidases and the products were chromatographed on Sephadex G-25 (0.6×24.5 cm column) equilibrated with 0.15 M NaCl, 0.01 M Tris, pH 7.5. Flow rate was 8 ml/h and fraction volume was 0.3 ml.

Chondroitin AC and ABC lyases (Miles-Yeda, Elkhart, Indiana) (41), and heparinase (purified from *Flavobacterium* [19]), were employed according to published methods. National Institutes of Health glycosaminoglycan reference standards were generously provided by J. A. Cifonelli, University of Chicago, Chicago, IL.

Nonlabeled glycosaminoglycans and degradation products were detected spectrophotometrically by the carbazole method of Bitter and Muir (3) with glucuronolactone as the standard.

Evaluation of Mast Cells for Accessory Cell and Natural Killer Function

Mast cell clones were examined as previously described for the ability to enhance Ig secretion (31) or to mediate NK lysis (29).

RESULTS

Ultrastructure

By TEM, clones MC/5 and MC/9 (Figs. 1 and 2B) consisted entirely of mast cells exhibiting various degrees of maturity, as judged both by nuclear and cytoplasmic criteria. Uncloned mast cell populations (Fig. 2A, C, and D) contained >99% mast cells. Mast cell nuclei were round with occasional nuclear membrane indentations, dispersed nuclear chromatin, and occasional small nucleoli. Rare cells exhibited bilobed nuclei. The plasma membrane displayed uniformly distributed, narrow short processes and folds. The cells' most prominent ultrastructural feature was cytoplasmic granules similar to those described in immature mast cells *in vivo* (4–6, 24) and in uncloned populations *in vitro* (5, 13, 51). These appeared to develop from large empty vacuoles in the active Golgi area (Fig. 3A). Vacuoles filled progressively with small vesicles (Figs. 2 and 3B) and then acquired dense matrix, some in the form of progranulelike (4–6, 24) structures (Figs. 2D, 3A and C). All cultures or clones contained at least a few very immature-appearing cells that were devoid of dense granule matrix

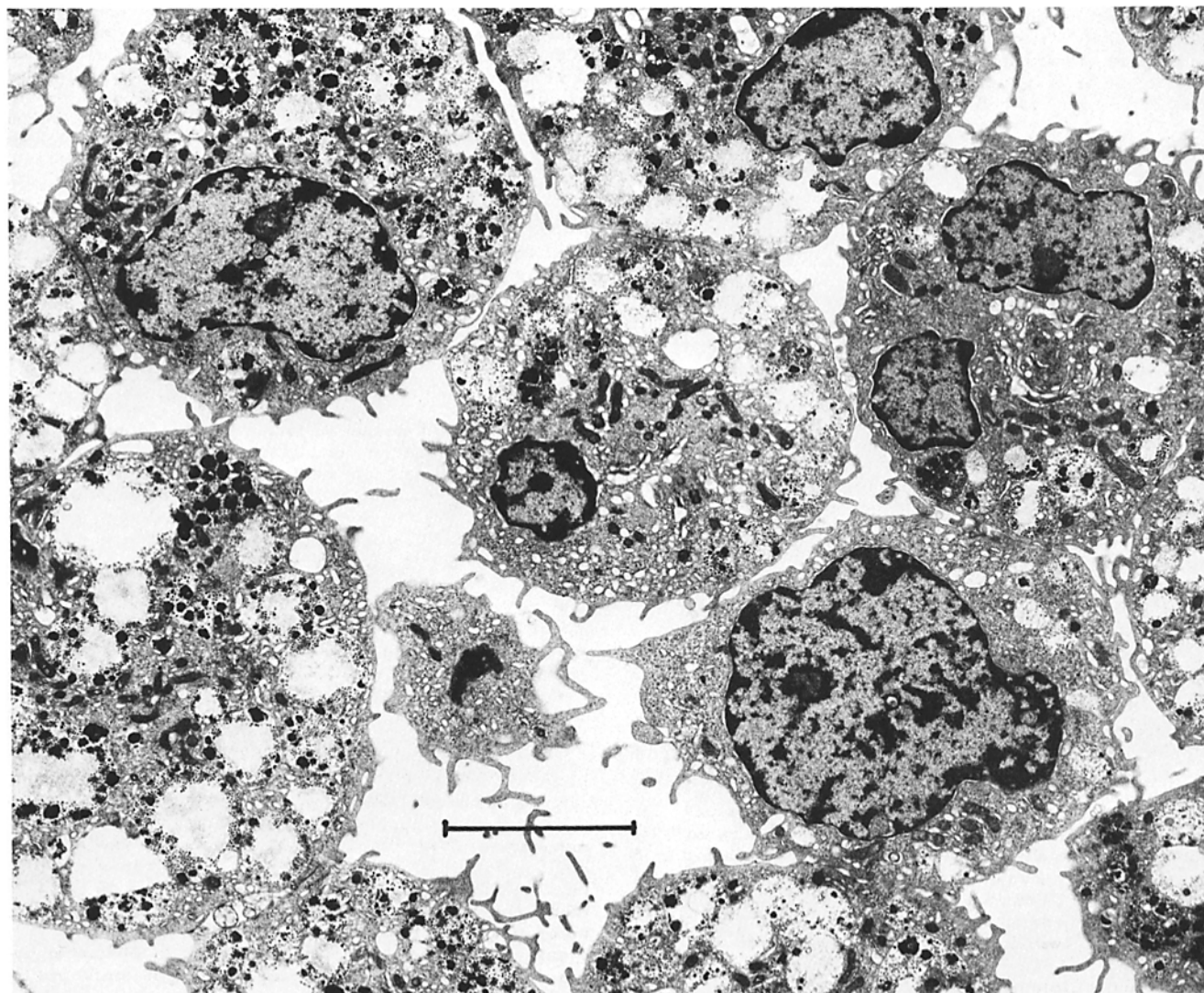


FIGURE 1 Typical immature mast cells from clone MC/5 derived from fetal liver. These mononuclear cells exhibit numerous immature granules that appear as large, membrane bounded, focally electron lucent structures containing small vesicles and/or dense progranules. Cells also contain mitochondria, cytoplasmic vesicles, and a prominent Golgi area. The cell surfaces are covered by short, narrow, uniform processes. Bar, 5 μm . $\times 2,000$.

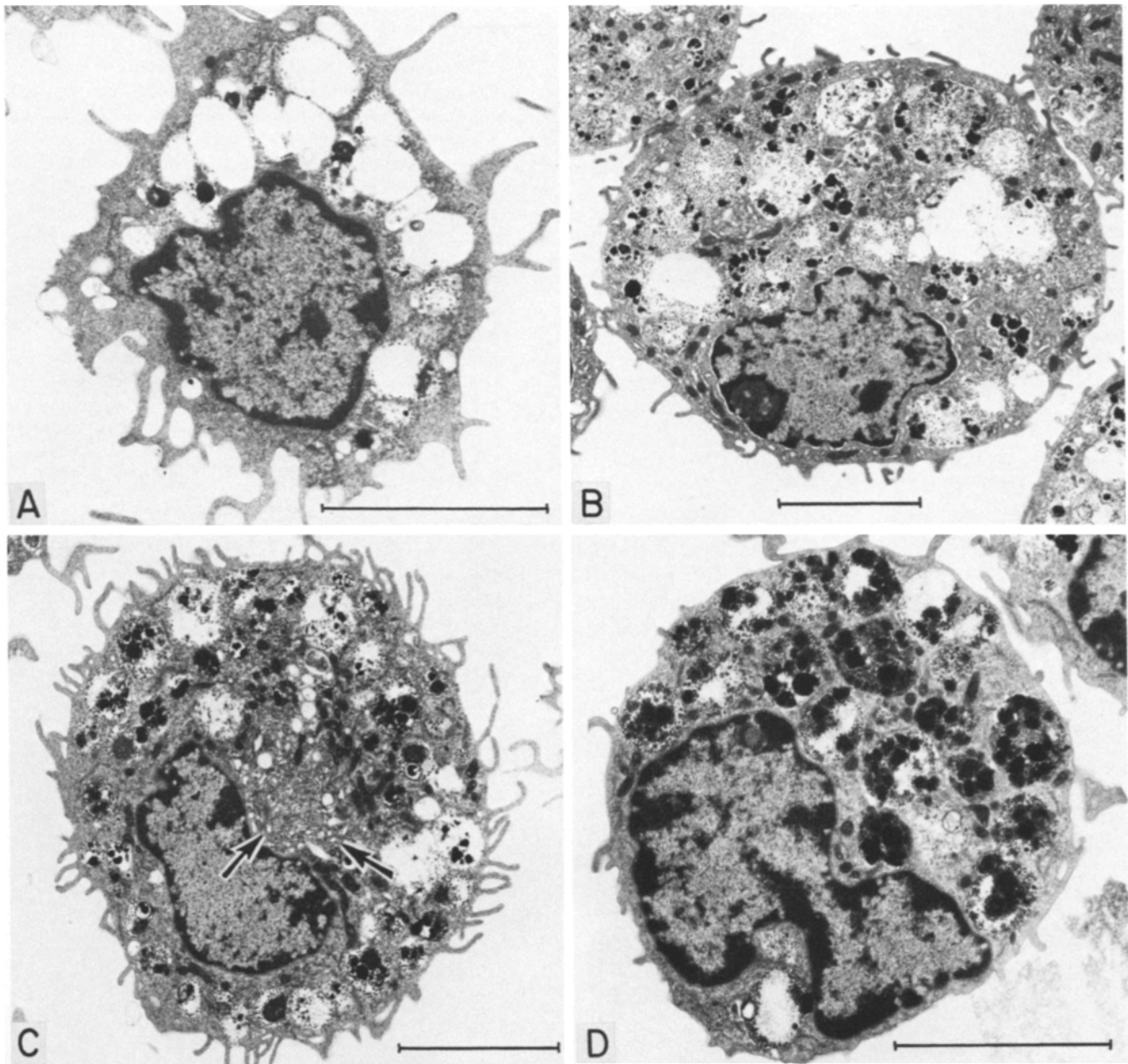


FIGURE 2 Mast cells from mouse bone marrow (line 3: A and C) fetal liver (MC/9: B), and spleen (line 1: D). The mast cells from bone marrow and spleen were maintained in WEHI-3 conditioned medium; MC/9 mast cells were maintained in Con A-CM. All cells contain a single eccentric nucleus and display short, narrow surface processes. The most immature cells (A) had cytoplasmic vacuoles containing only a few vesicles. In more mature cells (B, C), the cytoplasmic vacuoles acquired the features of immature mast cell granules. These contained variable proportions of fine, granular material; multiple small, round, extremely dense, progranules, and numerous small vesicles. The most mature cells (D) contained many granules rich in progranules. Although all populations contained cells of various maturity, line 3 appeared most immature and was comprised predominantly of cells like that shown in A. Vesicles and larger vacuoles appeared to originate in the active Golgi areas (indicated by arrows in C). Bars, 4 μ m. A, $\times 9,000$; B, $\times 5,500$; C, $\times 6,500$; D, $\times 8,500$.

and some were comprised predominantly of such cells (Fig. 2A). The cytoplasm also contained mitochondria and small vesicles. The cells had no cytoplasmic glycogen particles or aggregates, which are a feature of basophilic leukocytes and other granulocytes but not of mast cells.

Receptors for IgE and Histamine Content

Mast cell cultures (>99% mast cells by TEM) from bone marrow or spleen maintained with WEHI-3 conditioned me-

dium, and cloned mast cells (MC/9) from fetal liver maintained in Con A-CM or in 10% Con A-CM conditioned by Ly 1⁺2⁻ inducer T lymphocytes, expressed approximately half as many plasma membrane receptors for IgE as normal peritoneal mast cells (Table I). The MC/9 Fc ϵ R bound mouse IgE with an equilibrium constant at 37°C similar to that of mature mouse peritoneal mast cells (Fig. 4 and Table II). The histamine content of our mast cells was much less than that of mature peritoneal mast cells.

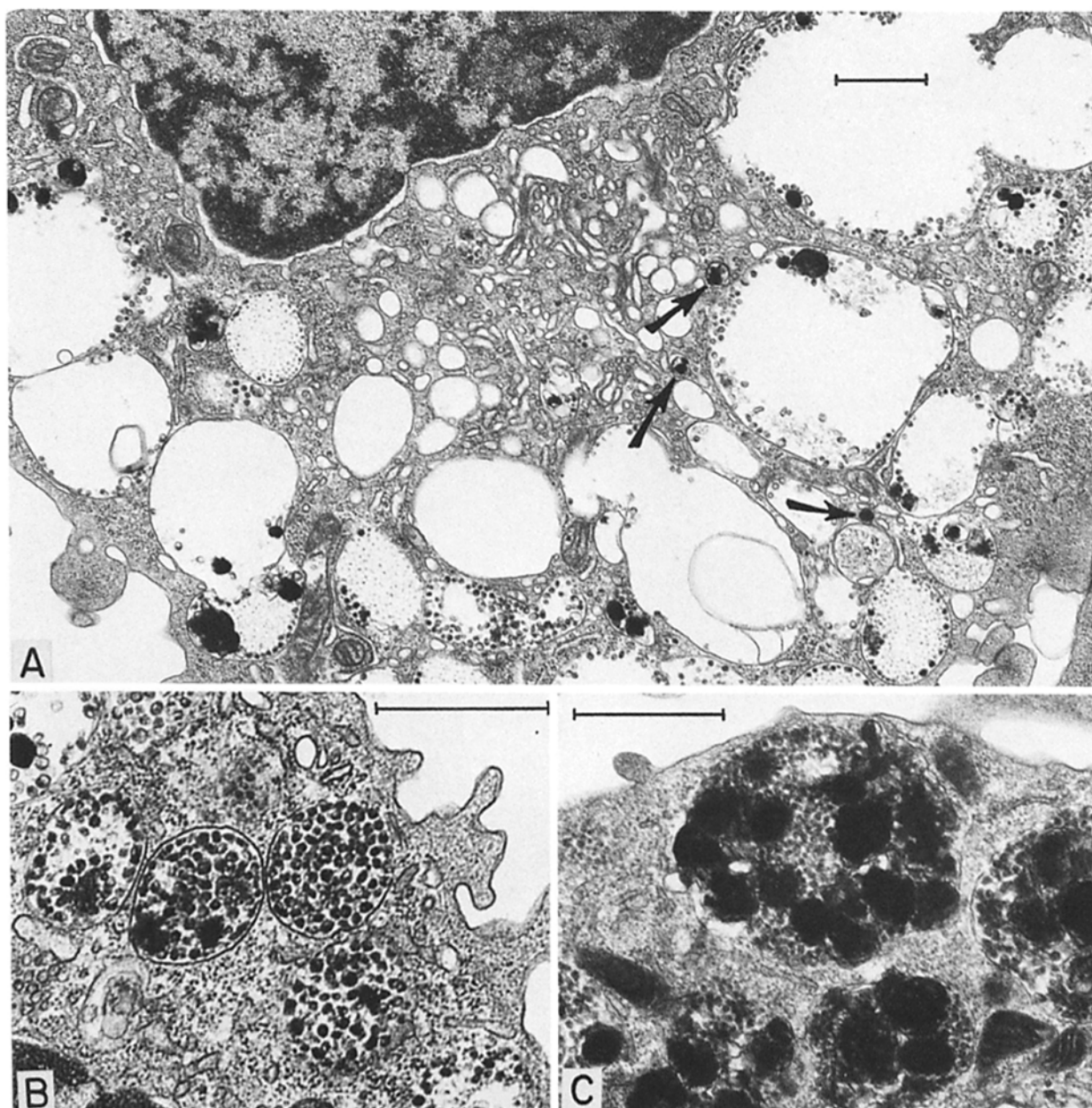


FIGURE 3 Mast cells from bone marrow (line 2: A and B) and spleen (line 1: C) maintained in WEHI-3 conditioned medium. The Golgi region in A contains numerous electron-lucent vesicles and vacuoles and occasional electron-dense progranules (arrows) adjacent to immature granules. Vesicle-rich immature granules are shown in B, and granules containing abundant dense matrix, in C. Bars, 1 μ m. A, $\times 14,000$; B, $\times 26,200$; C, $\times 22,800$.

Effect of Sodium Butyrate on Mast Cell Proliferation, Ultrastructure, and Histamine Content

Cloned mast cells (MC/9) were grown in Ly 1⁺2⁻ T lymphocyte-conditioned medium with or without 1 mM sodium butyrate. After 4 d, mast cells cultured without butyrate had increased sevenfold (8×10^5 to 5.4×10^6), contained 0.03 pg histamine/cell, and had a viability by trypan blue exclusion of 96%. By ultrastructure, these cells appeared very immature, with a few large cytoplasmic vacuoles that contained occasional small vesicles but no electron-dense granule matrix (Fig. 5A). By contrast, mast cells in 1 mM sodium butyrate ceased divid-

ing, remained viable (87–96% viable cells by trypan blue exclusion) and developed numerous cytoplasmic granules containing abundant electron-dense matrix material (Fig. 5B) similar to that of mature mast cells in vivo. Furthermore, butyrate-treated cells stored 50-fold more histamine (1.4 pg/cell) than untreated controls.

We investigated the effects of sodium butyrate over an ~ 100 -fold dose range (Table III). While mast cells grown with 1 mM butyrate exhibited profoundly diminished proliferation and markedly increased histamine content, mast cells grown in the presence of 0.3 mM butyrate were minimally affected. Sodium butyrate was toxic to cloned mast cells at concentrations ≥ 2 mM.

Sulfated Glycosaminoglycan Synthesis

CELLULAR LOCALIZATION OF INCORPORATED SODIUM [35 S] SULFATE: TEM autoradiography localized silver grains to the mast cells' cytoplasmic granules (Fig. 6). Although the majority of cells were labeled, the number of grains/cell varied widely. In general, the mast cells with the

TABLE I
Histamine Content and IgE Receptors ($Fc_\epsilon R$) of Mast Cell Lines

Cell*	Histamine \ddagger pg/cell	$Fc_\epsilon R \times 10^{-4}/\text{cell}\S$
Normal mouse peritoneal mast cells		
CBA/J	9.4–10.4	32.1 ± 7.3
BALB/c	5.2–10.6	23.0 ± 5.9
MC/9 mast cell clone (fetal liver)	0.1–0.4	12.5
Mast cell line 1 (spleen)	0.1–0.4	10.4
Mast cell line 2 (bone marrow)	0.1–0.7	7.0
Ly $1^{+}2^{-}/9$ (inducer T lymphocytes)	0	0
YAC-1 (mouse lymphoma)	ND	0

* Normal mouse peritoneal mast cells were purified to $\geq 88\%$ on a metrizamid gradient as described in reference 48. Cultured cells were maintained as described in the text.

\ddagger Range of 3 to 12 determinations performed over a period of weeks (spleen and bone marrow lines maintained in WEHI-3 conditioned medium) to a year (MC/9 clones maintained in Con A conditioned medium). Replicate simultaneous determinations of histamine content varied by $<10\%$.

\S Values for CBA/J or BALB/c mast cells are $M \pm \text{SEM}$ of five or six duplicate determinations, respectively. Values for cultured cells are means of duplicate determinations.

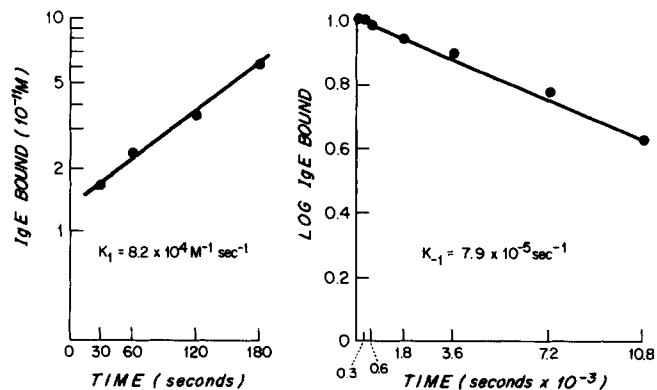


FIGURE 4 Forward rate constant (k_1) of IgE binding to MC/9 (left). Dissociation rate (k_{-1}) of IgE bound to MC/9 (right). The data were normalized by taking the amount of IgE bound at $t_0 = 1.0$. Each point is the mean of duplicate measurements.

TABLE II
Binding Characteristics of IgE to $Fc_\epsilon R$ on Cloned Mouse Mast Cells (MC/9) and Mouse or Rat Peritoneal Mast Cells*

Cell	k_1 $M^{-1}s^{-1} \times 10^5$	k_{-1} $s^{-1} \times 10^{-4}$	K_A $M^{-1} \times 10^9$
Cl. MC/9	0.82	0.79	1.04
Mouse (BALB/c) peritoneal mast cells (49)	1.94	1.11	1.75
Rat peritoneal mast cells (49)	1.65	0.21	7.86

* Association (k_1), dissociation (k_{-1}) and equilibrium (K_A) constants were determined as described in Materials and Methods.

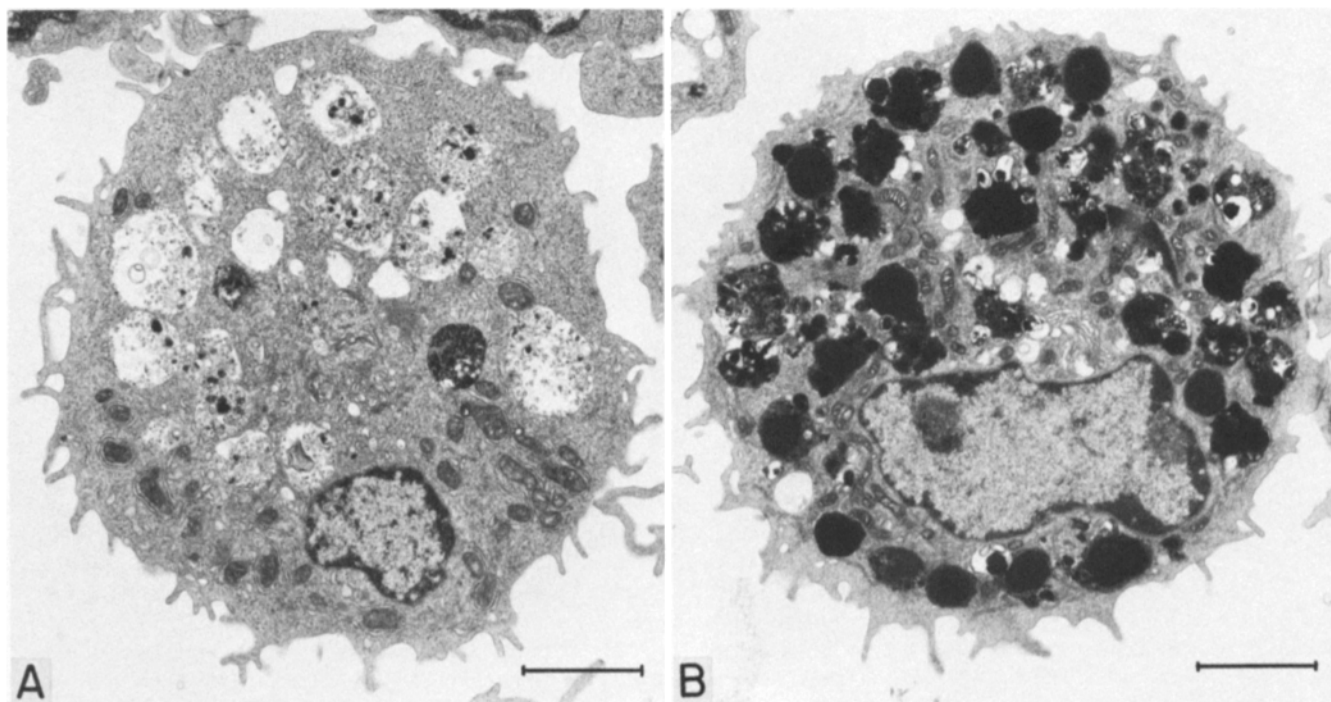


FIGURE 5 Cloned mast cells derived from fetal liver (MC/9) grown in Con A-CM supplemented with 15% Ly $1^{+}2^{-}$ inducer T lymphocyte-conditioned medium. The cell in A, cultured without sodium butyrate, contains cytoplasmic vacuoles with a few small vesicles but no electron-dense granule matrix. The cell in B, cultured for 4 d with 1 mM sodium butyrate, has numerous cytoplasmic granules rich in electron-dense granule matrix. Bars, $2 \mu\text{m}$. $\times 8,000$.

most mature granule morphology were most heavily labeled.

CHARACTERIZATION OF ^{35}S -GLYCOSAMINOGLYCANS. Assessment of the relative charge of the cloned mast cell (MC/9) ^{35}S -glycosaminoglycans, as determined by chromatography on DEAE cellulose, revealed a macromolecular peak which appeared coincident with the chondroitin sulfate reference standard (Fig. 7). Chromatography on Sepharose 4B established that the ^{35}S -glycosaminoglycans had an average M_r of 20,000 daltons (Fig. 8). These ^{35}S -macromolecules, and the chondroitin sulfate standard, were susceptible to complete hydrolysis to disaccharides by chondroitin ABC or AC lyase, but were insensitive to heparinase (Fig. 8), indicating that they repre-

TABLE III

Sodium Butyrate Decreases Mast Cell Proliferation and Increases Mast Cell Histamine Content

Sodium butyrate concentration	Doubling time	Histamine content
mM	h	pg/cell
0	20.4	0.07
0.01	20.5	0.06
0.04	20.5	0.06
0.11	21.5	0.06
0.33	25.8	0.08
1.00	132.0	0.50

Cloned mast cells (MC/9) were cultured for 4 d with or without sodium butyrate as described in the text.

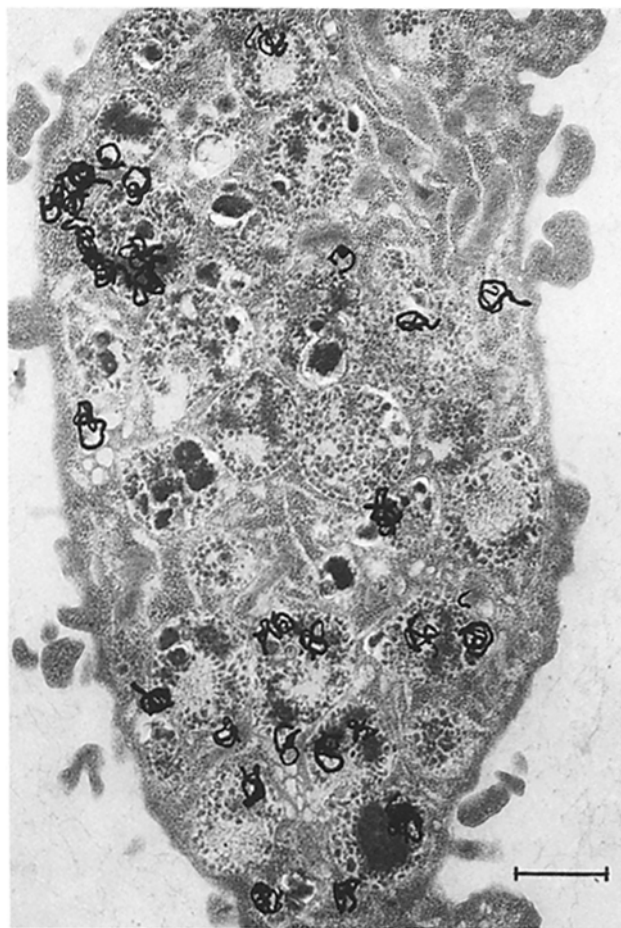


FIGURE 6 Electron microscopic autoradiography of ^{35}S -labeled immature granules in a cloned mouse mast cell (MC/9) after a 2-m exposure of emulsion. Most silver grains appear over the large, vesicle-rich cytoplasmic granules. Bar, 2 μm . $\times 6,000$.

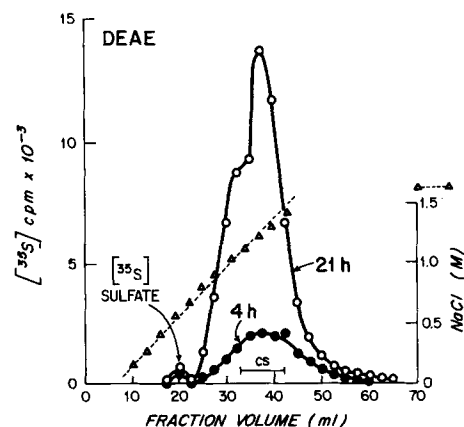


FIGURE 7 DEAE-Sephadex A-25 column (0.8 \times 6.9 cm) chromatography of pronase-digested cloned mast cells (MC/9) labeled with ^{35}S sulfate for 4 or 21 h (see text). The bar indicates the elution pattern of the chondroitin sulfate reference standard.

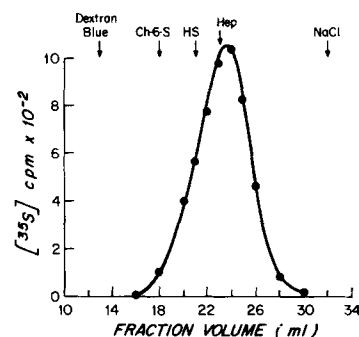


FIGURE 8 Sepharose 4B column (0.6 \times 110 cm) chromatography of pronase-digested cloned mast cells (MC/9) labeled with ^{35}S sulfate for 21 h (see text). The column was calibrated with Dextran Blue, National Institutes of Health reference standard glycosaminoglycans (Ch-6-S, chondroitin-6-sulfate; HS, heparan sulfate; Hep, heparin), and NaCl.

sented chondroitin ABC or AC lyase-sensitive chondroitin-sulfates without detectable heparin or dermatan sulfate (chondroitin sulfate B). Similar sulfated glycosaminoglycans were synthesized by mast cells incubated with $^{35}\text{SO}_4$ for 4, 12 (data not shown), and 21 h. By single dimensional electrophoresis (49), these molecules had charge characteristics similar to those of the chondroitins. Mast cells from spleen (line 1) or bone marrow (line 2 and line 3) also incorporated $^{35}\text{SO}_4$ into chondroitin sulfates, not heparin. MC/9 or bone marrow line 3 mast cells, cultured for 4 d with 1 mM sodium butyrate, synthesized ^{35}S -glycosaminoglycans identical to those of mast cells grown without butyrate, as judged by DEAE cellulose chromatography and susceptibility to enzymatic degradation. Although it did not induce synthesis of heparin, butyrate augmented mast cell storage of chondroitin sulfate. For example, butyrate-treated bone marrow mast cells (line 3) contained 81% more ^{35}S chondroitin sulfates after an 18-h incubation with $^{35}\text{SO}_4$ than untreated controls. Chromatographic (DEAE) analysis of mast cell culture medium failed to detect secreted ^{35}S -macromolecules.

In contrast to cloned mast cells, uncloned peritoneal mast cells incorporated $^{35}\text{SO}_4$ predominantly into heparin. These glycosaminoglycans eluted from DEAE cellulose as a single peak between 1.0 and 2.0 M NaCl. These ^{35}S -macromolecules, and the heparin reference standard, were susceptible to hydrolysis to disaccharides by heparinase but were insensitive to chondroitin ABC or AC lyase.

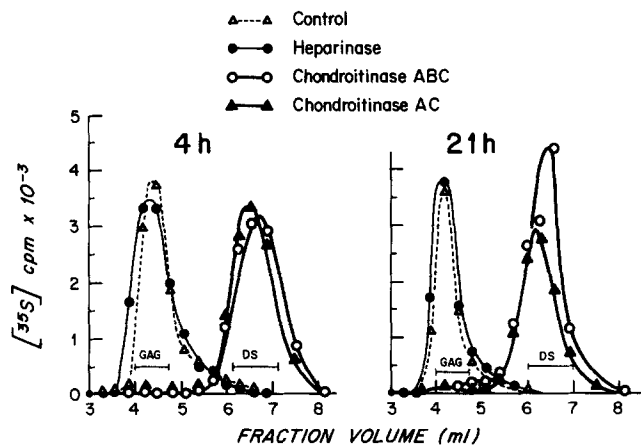


FIGURE 9 Sephadex G-25 column (0.6 × 24.5 cm) chromatography of aliquots of pronase-digested ³⁵S-labeled cloned mast cells (MC/9) without additional treatment (control) or after treatment with purified heparinase or chondroitinase ABC or AC lyase (chondroitinase ABC or AC). Bars indicate the elution patterns of native glycosaminoglycan standards (GAG) and their disaccharide degradation products (DS). The MC/9 macromolecules labeled with ³⁵S during either a 4- or 21-h incubation were degraded to disaccharides by chondroitinase ABC or AC lyase, but were unaffected by heparinase.

Accessory Cell Function and Natural Killer Lysis

Mast cells from three clones derived from fetal liver were unable to alter Ig secretion when added to enriched B lymphocytes. Data from a typical experiment are shown in Table IV. Analysis of mast cell clones with monoclonal antisera failed to detect Ia antigens.

Three mast cell clones from fetal liver or bone marrow did not lyse the YAC-1, EL-4, RL-12, and MBL-2 lymphomas and the P815 mastocytoma. A representative experiment is shown in Table V.

DISCUSSION

Cloned mast cells resembled by ultrastructure proliferating immature mast cells found in vivo (4–6, 24). Like immature mast cells, these clones contained cytoplasmic granules with numerous small vesicles and various amounts of electron dense progranulelike structures. Despite their immature appearance by ultrastructure and their low histamine content, cloned mast cells expressed nearly as many Fc_εR as mature peritoneal mast cells. In addition, the Fc_εR of cloned mast cells bound IgE with an equilibrium constant similar to that of normal mouse peritoneal mast cells. Uncoloned rat mast cells also express Fc_εR when their histamine content is very low (14), supporting the notion that incorporation of Fc_εR into the plasma membrane represents an early event in the program of mast cell differentiation. While some mast cell-like lines reportedly bear Ia antigens (42, 51), mouse (7, 52) and rat (28) mature peritoneal mast cells do not express Ia antigens detectable by alloantisera. We have not detected Ia antigens on the surface membranes of cloned mast cells by immunofluorescence with monoclonal reagents. Although expression may vary according to culture conditions, degree of differentiation (47), or source of antisera, we have not observed Ia expression at any time with the cells described in this report.

The relationship of mast cells to basophils has been widely discussed (12). Basophils are bone marrow-derived granulocytes that normally circulate in the blood and, unlike mast

cells, are not ordinarily found in connective tissues. Although basophils and mast cells express certain biochemical and functional similarities, they are not identical. Electron microscopy can be used to distinguish basophils from mast cells in all mammalian species, including the mouse (8, 10). Ultrastructurally, our cloned mast cells resemble immature mast cells found in vivo, not mouse basophils. Mouse basophils lack the uniform surface processes of normal and cloned mast cells, have a more central and polylobed nucleus, and have cytoplasmic granules that are fewer, larger, and different in ultrastructure than those of mast cells (10). Mouse tissue mast cells and granulocytes may share a common hematopoietic precursor (15), although the morphology of this precursor cell, and the factor(s) that control its division and differentiation, have not been described. We do not know whether our cloned cells are committed to the mast cell lineage alone; however, we have not observed the development of other cell types using the culture conditions described in this study.

We have recently reported that a cloned mouse cell line mediating natural killer lysis (29) resembles immature basophils by ultrastructure and expresses high affinity Fc_γR (11). In the present study, cloned immature mast cells were devoid of natural killer cell activity. We have also tested the ability of mast cell clones to regulate other immune functions. Mast cell clones do not enhance B cell Ig secretion. To date, we have not observed inhibition of Ig secretion by cloned mast cells (data not shown). However, histamine (37, 39) and perhaps other mediators released by mast cells may influence T lymphocyte activity, and we are currently testing whether Ig secretion is affected by cloned mast cells sensitized with IgE and degranulated with antigen.

We have been able to regulate mast cell proliferation and induce maturation in vitro. Cloned mast cells grown in medium conditioned by Ly 1⁺2⁺ T lymphocytes (see Materials and Methods) divided with a doubling time of ~20 h. These cells

TABLE IV
Mast Cells Do Not Activate B Cells to Secrete Ig

No. of T lymphocytes or mast cells × 10 ⁻⁴ /culture	No. of Ig-secreting cells after addition of	
	T lymphocytes	Mast cells (MC/9)
0	104	—
1	ND	42
2	ND	65
5	ND	117
10	1053	ND
20	1313	ND
25	ND	39

Ly 1⁺ T lymphocytes or cloned mast cells were added to 5 × 10⁶ B cells and incubated for 5 d. ND, not done.

TABLE V
Mast Cells Do Not Mediate NK Lysis

Effector:Target	Specific lysis mediated by		
	NK-1	NK-2	MC/9
	%	%	%
10:1	36	39	0
5:1	38	40	0
2.5:1	35	34	0

Mast cells (MC/9) or cells with natural killer function (NK-1, NK-2) were incubated with ⁵¹Cr-labeled YAC-1 lymphoma targets for 3.5 h.

appeared very immature by ultrastructure and contained only a few cytoplasmic vacuoles devoid of electron-dense granule matrix. Their histamine content was very low. By contrast, mast cells grown in the same medium supplemented with 1 mM sodium butyrate stopped dividing, developed more mature cytoplasmic granules containing increased levels of ^{35}S -glycosaminoglycans, and stored up to 50 times more histamine than control mast cells without butyrate. These findings are consistent with earlier work identifying the cytoplasmic granule as the site of histamine storage in mature mast cells (1).

The cloned mast cells described in this report may represent a subset which grows under selective culture conditions. Although there is no proof that mast cells in vivo are comprised of distinct subpopulations, variations in the morphology and histochemistry of mast cells in different tissues have suggested this possibility. For example, microspectrophotometric analysis suggests that the sulfated glycosaminoglycans of rat intestinal mucosal mast cells, like those of our clones, are similar to chondroitin sulfates (50). Mucosal mast cells also resemble mast cell clones in their low histamine content (2, 25) and responsiveness to T lymphocyte regulation (21, 33, 40). However, mouse mucosal mast cells have not been isolated, and their glycosaminoglycans have not been analyzed by hydrolysis with purified mucopolysaccharidases. The number and IgE binding affinity of their Fc ϵ R have not been determined. Although the extent and causes of mast cell phenotypic variation remain to be fully defined, some differences among mast cell populations may be regulated by microenvironmental factors affecting morphology, mediator content, or maturation. In this view, the ultrastructure and mediator content of cloned mast cells may reflect their immaturity or the influence of their culture conditions, rather than their derivation from a distinct mast cell subpopulation. In support of this notion, histochemical evidence has suggested that, like mucosal mast cells, immature connective tissue mast cells in vivo contain little or no heparin (6). In addition, cloned mast cells resemble immature connective tissue mast cells by ultrastructure (4–6, 24). On the other hand, certain mastocytoma sublines reportedly synthesize heparin, albeit in small quantities, while they are rapidly dividing and their mediator content is very low (27). By contrast, we have not detected heparin synthesis in our cloned mast cells even after using butyrate to halt proliferation and induce maturation. Whether other agents may induce further maturation and synthesis of heparin remains to be determined.

We thank A. Sterk for excellent technical assistance.

These studies were supported by U. S. Public Health Service grants CA 28834, AI 16925, GM 07753, AI 12184, AI 13600, AI 10060, and HL 28625. J. A. Marcum was supported by American Heart Association Fellowship No. 13-420-801. This is publication No. 474 from the O'Neill Laboratories at the Good Samaritan Hospital.

REFERENCES

- Anderson, P., and B. Uvnäs. 1975. Selective localization of histamine to electron dense granules in antigen-challenged sensitized rat mast cells and to similar granules isolated from sonicated mast cells. An electron microscope autoradiographic study. *Acta Physiol. Scand.* 94:63–73.
- Befus, A. D., F. L. Pearce, J. Gaudie, P. Horwood, and J. Bienenstock. 1982. Mucosal mast cells. I. Isolation and functional characteristics of rat intestinal mast cells. *J. Immunol.* 128:2475–2480.
- Bitter, T., and H. M. Muir. 1962. A modified uronic acid carbazole reaction. *Anal. Biochem.* 4:330–334.
- Combs, J. W. 1966. Maturation of rat mast cells: an electron microscopic study. *J. Cell Biol.* 31:563–575.
- Combs, J. W. 1971. An electron microscopic study of mouse mast cells arising in vivo and in vitro. *J. Cell Biol.* 48:676–684.
- Combs, J. W., D. Lagunoff, and E. P. Benditt. 1965. Differentiation and proliferation of embryonic mast cells of the rat. *J. Cell Biol.* 25:577–592.
- Daéron, M., and G. A. Voisin. 1979. Mast cell membrane antigens and Fc receptors in anaphylaxis. I. Products of the major histocompatibility complex involved in alloantibody-induced mast cell activation. *Immunology.* 38:447–458.
- Dvorak, A. M. 1978. Biology and morphology of basophilic leukocytes. In *Immediate Hypersensitivity: Modern Concepts and Developments*. M. K. Bach, editor. Marcel Dekker, NY 369–405.
- Dvorak, A. M., S. J. Galli, E. Morgan, A. S. Galli, and H. F. Dvorak. 1981. Anaphylactic degranulation of guinea pig basophilic leukocytes. I. Fusion of granule membranes and cytoplasmic vesicles: formation and resolution of degranulation sacs. *Lab. Invest.* 44:174–191.
- Dvorak, A. M., G. Nabel, K. Pyne, H. Cantor, H. F. Dvorak, and S. J. Galli. 1982. Ultrastructural identification of the mouse basophil. *Blood.* 59:1279–1285.
- Galli, S. J., A. M. Dvorak, T. Ishizaka, G. Nabel, H. Der Simonian, H. Cantor, and H. F. Dvorak. 1982. A cloned cell with natural killer function resembles basophils by ultrastructure and expresses IgE receptors. *Nature (Lond.)* 298:288–290.
- Galli, S. J., and H. F. Dvorak. 1979. Basophils and mast cells: structure, function, and role in hypersensitivity. In *Cellular, Molecular, and Clinical Aspects of Allergic Diseases*. S. Gupta and R. A. Good, editors. Plenum, NY, 1–53.
- Ginsburg, H., I. Nir, I. Hammel, R. Eren, B.-A. Weissman, and Y. Naot. 1978. Differentiation and activity of mast cells following immunization in cultures of lymph node cells. *Immunology.* 35:485–502.
- Ishizaka, T., T. Adachi, T.-H. Chang, and K. Ishizaka. 1977. Development of mast cells in vitro. II. Biologic function of cultured mast cells. *J. Immunol.* 118:211–217.
- Kitamura, Y., M. Yokoyama, H. Matsuda, and T. Ohno. 1981. Spleen colony-forming cell as common precursor for tissue mast cells and granulocytes. *Nature (Lond.)* 291:159–160.
- Klein, J., L. Flaherty, J. L. Vandeberg, and D. C. Shreffler. 1978. H-2 haplotypes, genes, regions, and antigens: first listing. *Immunogenetics.* 6:489–512.
- Kulczycki, A., Jr., and H. Metzger. 1974. The interaction of IgE with the rat basophilic leukemia cells. II. Quantitative aspects of the binding reaction. *J. Exp. Med.* 140:1676–1695.
- Lewis, R. G., A. F. Spencer, and J. E. Silbert. 1973. Biosynthesis of glycosaminoglycans by cultured mastocytoma cells. *Biochem. J.* 134:455–463.
- Linker, A., and P. Hovingh. 1972. Heparinase and heparitinase from *Flavobacterium*. *Methods Enzymol.* 28:902–911.
- Liu, F.-T., J. W. Bohn, E. L. Ferry, H. Yamamoto, C. A. Molinaro, L. A. Sherman, N. R. Klinman, and D. H. Katz. 1980. Monoclonal dinitrophenol-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728–2737.
- Mayrhofer, G. 1980. Thymus-dependent and thymus-independent subpopulations of intestinal intraepithelial lymphocytes: a granular subpopulation of probable bone marrow origin and relationship to mucosal mast cells. *Blood.* 55:532–535.
- Metcalfe, D. D., C. E. Bland, and B. Sredni. 1981. Comparison of proteoglycans synthesized during lymphocyte-dependent mast cell and basophil proliferation. *Fed. Proc.* 40:1023.
- Metcalfe, D. D., R. A. Lewis, J. E. Silbert, R. D. Rosenberg, S. I. Wasserman, and K. F. Austen. 1979. Isolation and characterization of heparin from human lung. *J. Clin. Invest.* 64:1537–1543.
- Miller, H. R. P. 1971. Immune reactions in mucous membranes. II. The differentiation of intestinal mast cells during helminth expulsion in the rat. *Lab. Invest.* 24:339–347.
- Miller, H. R. P., and R. Walshaw. 1972. Immune reactions in mucous membranes. IV. Histochemistry of intestinal mast cells during helminth expulsion in the rat. *Am. J. Pathol.* 69:195–208.
- Mori, Y., H. Akedo, K. Tanaka, Y. Tanigaki, and M. Okada. 1979. Effect of sodium butyrate on the granulogenesis of mastocytoma cells. *Exp. Cell Res.* 118:15–22.
- Mori, Y., H. Akedo, Y. Tanigaki, K. M. Tanaka, M. Okada, and N. Nakamura. 1980. Effect of sodium butyrate on the production of serotonin, histamine, and glycosaminoglycans by cultured murine mastocytoma cells. *Exp. Cell Res.* 127:465–470.
- Mossmann, H., W. Lauer, U. Vortisch, E. Gunther, H. Bazin, and D. K. Hammer. 1976. Stimulation and suppression of rat mast cell functions by alloantibodies. *Eur. J. Immunol.* 6:591–594.
- Nabel, G., L. R. Bucalo, J. Allard, H. Wigzell, and H. Cantor. 1981. Multiple activities of a cloned cell line mediating natural killer function. *J. Exp. Med.* 153:1582–1591.
- Nabel, G., S. J. Galli, A. M. Dvorak, H. F. Dvorak, and H. Cantor. 1981. Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells. *Nature (Lond.)* 291:332–334.
- Nabel, G., J. S. Greenberger, M. A. Sakakeeny, and H. Cantor. 1981. Multiple biologic activities of a cloned inducer T-cell population. *Proc. Natl. Acad. Sci. U. S. A.* 78:1157–1161.
- Nagao, K., K. Yokoro, and S. A. Aaronson. 1981. Continuous lines of basophil/mast cells derived from normal mouse bone marrow. *Science (Wash. D. C.)* 212:333–335.
- Nawa, Y., and H. R. P. Miller. 1979. Adoptive transfer of the intestinal mast cell response in rats infected with *Nippostrongylus brasiliensis*. *Cell Immunol.* 42:225–239.
- Ogren, S., and U. Lindahl. 1971. Degradation of heparin in mouse mastocytoma tissue. *Biochem. J.* 125:1119–1129.
- Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ia allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol.* 7:115–129.
- Orenstein, N. S., S. J. Galli, A. M. Dvorak, J. E. Silbert, and H. F. Dvorak. 1978. Sulfated glycosaminoglycans of guinea pig basophilic leukocytes. *J. Immunol.* 121:586–592.
- Plaut, M., L. M. Lichtenstein, E. Gillespie, and C. S. Henney. 1973. Studies on the mechanism of lymphocyte-mediated cytotoxicity. IV. Specificity of the histamine receptor on effector T cells. *J. Immunol.* 111:389–394.
- Razin, E., C. Cordon-Cardo, and R. A. Good. 1981. Growth of a pure population of mouse mast cells in vitro with conditioned medium derived from concanavalin A-stimulated splenocytes. *Proc. Natl. Acad. Sci. U. S. A.* 78:2559–2561.
- Rocklin, R. E., D. Greineder, and K. L. Melmon. 1979. Histamine induced suppressor factor (HSF): further studies on the nature of the stimulus and the cell which produces it. *Cell Immunol.* 44:404–415.
- Ruitenberg, E. J., and A. Elgersma. 1976. Absence of intestinal mast cell response in congenitally athymic mice during *Trichinella spiralis* infection. *Nature (Lond.)* 264:258–260.
- Saito, H., T. Yamazata, and S. Suzuki. 1968. Enzymatic methods for the determination of small quantities of isomeric chondroitin sulfate. *J. Biol. Chem.* 243:1536–1542.
- Schrader, J. W. 1981. The in vitro production and cloning of the P cell, a bone-marrow derived null cell that expresses H-2 and Ia-antigen, has mast-cell-like granules, and is regulated by a factor released by activated T cells. *J. Immunol.* 6:452–458.
- Schrader, J. W., S. J. Lewis, I. Clark-Lewis, and J. G. Culvenor. 1981. The persisting (P) cell: histamine content, regulation by a T cell-derived factor, origin from a bone marrow precursor, and relationship to mast cells. *Proc. Natl. Acad. Sci. U. S. A.* 78:323–327.
- Shaff, R. E., and M. A. Beaven. 1979. Increased sensitivity of the enzymatic isotopic assay of histamine: measurement of histamine in plasma and serum. *Anal. Biochem.* 94:425–430.

45. Shen, F.-W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics*. 2:591-595.
46. Siraganian, R. P., and W. A. Hook. 1980. Histamine release and assay methods for the study of human allergy. In *Manual of Clinical Immunology* Second Edition. N. R. Rose and H. Friedman, editors. American Society for Microbiology, Washington, D.C. 808.
47. Steinman, R. M., N. Nogueira, M. D. Witmer, J. D. Tydings, and I. S. Mellman. 1980. Lymphokine enhances the expression and synthesis of Ia antigens on cultured mouse peritoneal macrophages. *J. Exp. Med.* 152:1248-1261.
48. Sterk, A., and T. Ishizaka. 1982. Binding properties of IgE receptors on normal mouse mast cells. *J. Immunol.* 128:838-843.
49. Stevens, R. L., M. Columbo, J. J. Gonzales, W. Hollander, and K. Schmid. 1976. The glycosaminoglycans of the human artery and their changes in atherosclerosis. *J. Clin. Invest.* 58:470-481.
50. Tas, J., and R. G. Berndsen. 1977. Does heparin occur in mucosal mast cells of the rat small intestine? *J. Histochem. Cytochem.* 25:1058-1062.
51. Tertian, G., Y.-P. Yung, D. Guy-Grand, and M. A. S. Moore. 1981. Long-term in vitro culture of murine mast cells. I. Description of a growth-factor-dependent culture technique. *J. Immunol.* 127:788-794.
52. Unanue, E. R., M. E. Dorf, C. S. David, and B. Benacerraf. 1974. The presence of I-region associated antigens on B cells in molecules distinct from immunoglobulin and H-2K and H-2D. *Proc. Natl. Acad. Sci. U. S. A.* 71:5014-5016.
53. Yung, Y.-P., R. Eger, G. Tertian, and M. A. S. Moore. 1981. Long-term in vitro culture of murine mast cells. II. Purification of a mast cell growth factor and its dissociation from TCGF. *J. Immunol.* 127:794-799.
54. Yurt, R. W., R. W., Leid, Jr., K. F. Austen, and J. E. Silbert. 1977. Native heparin from rat peritoneal mast cells. *J. Biol. Chem.* 252:518-512.